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ANALYSIS OF STARCH SYRUP[1]

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Abstract

Examination of a number of starch syrups revealed wide differences in content of non-carbohydrates: water, 17.4 to 28.7%; protein 0.05 to 1.0%; ash 0.2 to 1.6%, sodium chloride 0.1 to 0.7% and sulphur dioxide 0 to 340 p.p.m. The commercial wheat syrups examined had high protein and salt contents, and a sample prepared by direct acid hydrolysis also contained an appreciable amount of ammonium chloride. Maximum absorption by storage color was observed at 3500 to 4500 Å; this region is therefore suitable for routine color measurements. Steinhoff's empirical copper acetate procedure for the direct determination of dextrose was found to be strongly influenced by heat treatment, total salt, and copper concentration; it was not improved by changing the composition of the reaction mixture. Moisture data obtained by vacuum oven and toluol distillation methods showed close agreement on syrups of widely different dextrose equivalent value; an improved stirring device is described for the incorporation of syrup and dispersing agent in either of these procedures.

Introduction

Preliminary to an investigation of starch syrup production (6), a study was made of analytical methods and of the composition of commercial products. Observations on factors influencing the analytical determinations and modifications that proved advantageous are described in this paper. Data also are presented on the composition of domestic and imported corn, wheat, and potato syrups that were marketed during the past four years.

Methods of Analysis

Sodium chloride contents of the ashed samples were estimated from sodium determinations with magnesium uranyl acetate (1). Sulphur dioxide was determined on diluted (1 : 1) 50 gm. syrup samples by adding alkali and titrating with iodine (11). Optical rotatory power was determined at 20° C. on 100 ml. solutions containing 26 gm. solids, and was expressed as 'specific rotation' on a total solids basis. Dextrose equivalent, also expressed on a solids basis, was determined by Zerban and Sattler's modification of the Shaffer–Hartmann method (25).

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Total Nitrogen

The digestion proceeded satisfactorily and quantitative nitrogen recoveries were obtained when 5.0 to 7.5 gm. syrup solids and 45 cc. sulphuric acid were employed in the Kjeldahl nitrogen determinations. These conditions are considered superior to those that have been prescribed previously (9, 22). The accuracy of the determination suffers from the use of 1 to 2 gm. samples (22), particularly when dealing with high quality syrups having total nitrogen contents of 10 to 20 mgm. %. With 10 gm. samples (9), the digestion mixture is very apt to solidify before clearing.

Ammonia Nitrogen

Folin's aeration method (10) proved fairly satisfactory when adapted as follows. Ten grams syrup solids was dissolved in 50 ml. water and transferred to a Kjeldahl flask. Ammonia-free air was drawn through the solution for one-half hour. Two grams of sodium carbonate was added and the solution was aerated at the rate of 30 liters per hour for five hours. During aeration, the liquid temperature was maintained between 35° and 40° C. The liberated ammonia was absorbed in $N/140$ sulphuric acid. Recoveries with 1 and 3 mgm. additions of ammonium chloride in the presence of 10 gm. starch syrup were 90 and 95% respectively.

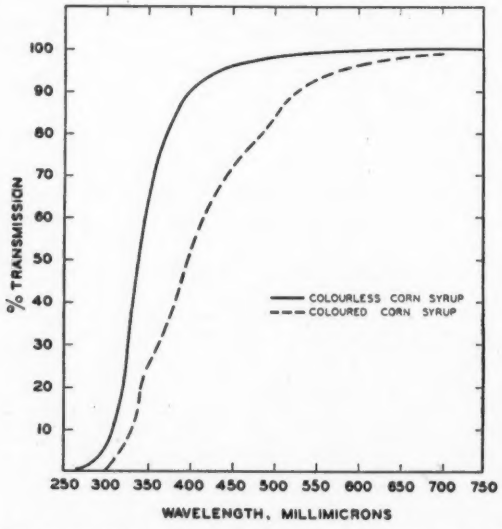

FIG. 1. *Spectral transmittance of freshly prepared 42° Bé. corn syrup (50 D.E.) and of syrup that had developed color during storage.*

Color

The transmission curves of Fig. 1 were determined with a Beckman spectrophotometer against distilled water blanks. Absorption was high at the blue and low at the red end of the visible spectrum with both the colored

and colorless syrups (unblended), and neither syrup showed definite absorption bands. Similar characteristics have been reported by Wayne (23) for cane molasses, caramel solutions, and raw cane sugar solutions. Since the largest difference in transmission between the colored and colorless starch syrups was shown between 350 and 450 mμ, Filter 420 was selected for subsequent color measurements with the Evelyn colorimeter (6). A syrup of corresponding density that had been rendered colorless with activated carbon was used as a standard.

The Copper Acetate Procedure for Dextrose

The copper acetate procedure developed by Steinhoff (21), in which 50% sodium acetate solution replaces Fehling's II, has attracted interest as a convenient means of determining the actual dextrose content of starch syrups (19, 20, 25). Later modifications (20, 25) are chiefly concerned with the determination of the reduced copper rather than with the reaction itself. The procedure suffers from its relative insensitivity to dextrose contents of from 40 to 100 mgm. (Fig. 2) and agreement between duplicates is usually

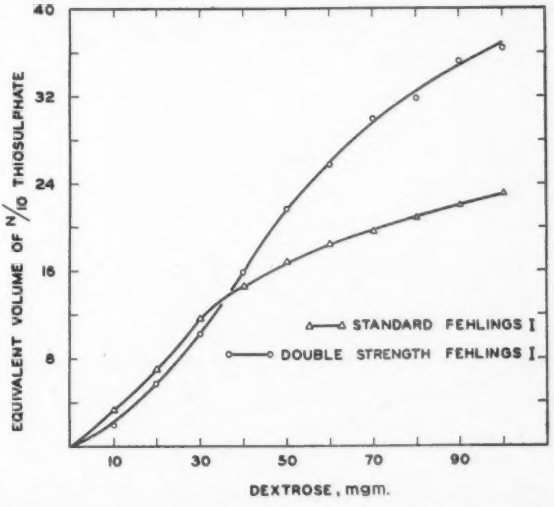

FIG. 2. *Effect of increased copper content on the reduction of Steinhoff's copper acetate solution by 10 to 100 mgm. dextrose (30 min. at 100° C.).*

poorer than is obtained in routine applications of copper tartrate procedures. The literature (19, 20, 21, 25) revealed a complete lack of information on the effects of heating time and composition of the reaction mixture on copper reduction by dextrose.

A plateau in copper reduction is not attained either after 20 min. or longer periods in the boiling water bath.

Time at 100° C., min.:	5	10	15	20	30	40	60
Equivalent volume of $N/10$ $Na_2S_2O_3$ for 50 mgm. dextrose, ml.	6.57	13.0	15.4	16.2	16.7	17.2	17.5

Differences in wall thickness between flasks, in bath temperature, and in agitation cannot be avoided entirely, and the resulting variations in heat treatment may be expected to influence the results.

When the usual 50 ml. volume is maintained, reduction of Steinhoff's copper acetate solution by dextrose is strongly depressed by the addition of either sulphuric acid or sodium hydroxide (Fig. 3). These observations signify that this procedure, in common with the ferricyanide and copper methods for reducing sugars examined by Weintraub and Price (24), is strongly influenced by initial pH and salt content.

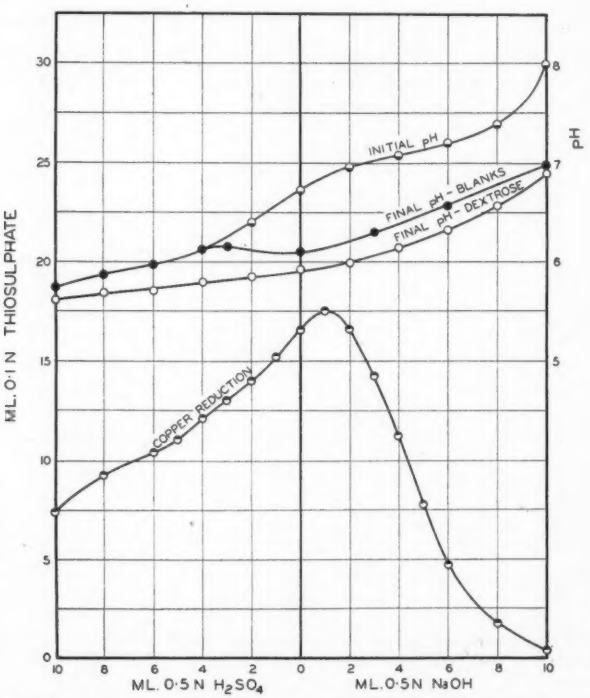

FIG. 3. *Effect of alkali and acid additions on the reduction of Steinhoff's copper acetate solution by 50 mgm. dextrose (30 min. at 100° C.).*

It has been claimed (20, 21) that no change in pH occurs during reduction of the copper acetate reagent, since free acetic acid is not present as in the older Barfoed reagent. Fig. 3 shows that an appreciable decrease occurs, and that the pH change is greater in the presence than in the absence of dextrose.

Doubling the copper content of the reaction mixture effects drastic changes (Fig. 2). With 40 to 100 mgm. dextrose, copper reduction was increased by as much as 50%, but copper reduction was slightly diminished at lower dextrose contents. The data were more erratic at high dextrose concentrations when double strength Fehling's I was employed, irrespective of the acetate level. Interference by maltose was also increased. For example, copper reduction by 50 mgm. maltose in the presence of 50 mgm. dextrose was increased from the equivalent of 0.74 ml. $N/10$ sodium thiosulphate at the usual copper concentration to 1.64 ml. when the copper concentration was doubled.

Considerable exploratory work indicated that the copper acetate procedure is not improved by changing the composition of Steinhoff's reaction mixture. Corrections were not made for the slight reducing action of maltose in routine applications because of existing uncertainty respecting the magnitude of its effect and the present lack of agreement concerning the actual maltose content of typical starch syrups (12, pp. 269-270; 18). Some improvement in reproducibility was indicated with 30-min. heating periods, but the procedure otherwise was applied in accordance with earlier directions (25).

Moisture

With proper precautions, the water content of starch syrup may be approximated with a hydrometer spindle (7, 8) or refractometer (5, 13) but the extremely high viscosity of syrup is a troublesome feature in both methods. The density and refractivity of starch syrups also are considerably influenced by degree of hydrolysis and salt content.

The chief obstacles in direct determinations of water content are the vitreous state that is attained before water is completely removed, high initial viscosity which impedes uniform distribution on dispersing agents, and the uncertain effects of impurities on sugar decomposition. The direct methods that have been proposed differ chiefly in the means provided for sample dispersion. Drying on quartz sand, pumice, and coiled filter paper was tested, but in our experience was less satisfactory than oven and distillation methods in which Filter-cel is employed.

The distillation method prescribed for '70' and '80' corn sugar by Cleland and Fetzer (4) was employed with minor changes. Toluol was substituted for benzene. A correction of 0.04 ml. per 100 ml. toluol was applied for water solubility. Anhydrous Hyflo Supercel was employed as the dispersing agent. The syrup, dispersant, and toluol were worked to a uniform mass with the stirring device shown in Fig. 4. The handle of the stirrer was then detached, leaving the pestle in the distillation flask. The stirring device (Fig. 4) is made from 12/30 S/T joints, and in our experience has been more satisfactory than any device that has previously been prescribed for this purpose.

After the distillation apparatus was assembled, the flasks were placed in a Stanolax* oil bath having an initial temperature of 137° C. The bath temperature was adjusted to 132° to 133° C. as boiling began, and was subsequently maintained at this temperature. The moisture traps were immersed in a 20° C. bath for one hour before volume readings were taken. (Frozen

FIG. 4. *Improved stirrer for use in the determination of moisture in syrups.*

joints were freed by tapping with a graphite block.) Additional precautions with respect to the care of the distillation equipment were observed as recommended elsewhere (17).

It was found that appreciable water was recovered after a six hour distillation, and that distillation was complete in 12 to 16 hr.

Sample	Distillation time with toluol, hr.				
	4.5	6.5	12	16	22
	Apparent moisture content, %				
I	18.45 18.81	18.82 19.03	19.05 19.21	19.10 19.21	19.10 19.21
II	19.11 18.85	19.22 18.97	19.38 19.32	19.38 19.32	19.38 19.32

The higher boiling point of toluol did not reduce the distillation time for syrup appreciably below that required in distilling moisture from corn sugars with benzene (4). Less time apparently is required for the complete removal of water from starch syrups by toluol distillation than from 10 mesh and 40 mesh corn meal (15, 16).

* *Stanolax was found superior to glycerol or corn oil as bath liquid for the distillations. After the first day's use, smoking was negligible and there was no formation of tarry deposits.*

The Filter-cel vacuum oven method for starch syrup described by Cleland and Fetzer (3) was adopted for routine applications with minor changes, employing a drying period of 20 to 24 hr. (See "Adaptation of Filter-cel method" (3, p. 859.)) The stirring device described above was used in mixing the diluted syrup with the Filter-cel. The diluted samples were dried for two and one-half to three hours in a 100° C. air oven before transferring to the 100° C. vacuum oven. The vacuum oven was operated at approximately 15 mm. mercury and was equipped with the drying train prescribed by Sair and Fetzer (15).

The apparent moisture contents reported in the accompanying table were observed by vacuum oven drying of a syrup that showed 21.0% moisture by a 20 hr. distillation with toluol.

Moisture dish size, in.	Drying time *in vacuo* at 100° C., hr.							
	4	8	12	16	20	24	28	32
	Apparent moisture content, %							
2½ by ¾	20.33 20.01	20.68 20.68	20.73 20.75	20.83 20.83	20.85 20.85	20.85 20.85	20.89 20.90	20.93 20.94
3½ by 2		20.84 20.98	20.89 20.96	20.91 20.96	20.98 21.01	20.98 21.03	20.99 21.03	21.02 21.06

Extension of the drying period from 16 to 32 hr. resulted in a very gradual loss in weight, averaging less than 0.01% per hour. The values obtained by drying periods of 16 to 32 hr. *in vacuo* agreed with the 20 hr. toluol distillation value within 0.15%. A small but consistent difference in apparent moisture content was associated with the size of the drying dishes. This result may have been fortuitous, but it at least indicates that drying is more rapid and complete from the shallow syrup-Filter-cel masses of the larger dishes. The larger dish size also was more convenient and was therefore adopted for routine use.

Agreement between the toluol distillation and vacuum oven methods was not affected by degree of conversion between 30 and 70 D.E. The largest differences between duplicates were observed with the distillation method on extremely viscous syrups containing less than 18% water. In routine applications, agreement between duplicates usually was within 0.05% by the oven method and 0.2% by toluol distillation.

Composition of Commercial Starch Syrups

The syrups of Table I were obtained from commercial sources. The table corn syrups were of the usual golden type prepared by blending refiners' syrup (sucrose) and starch syrup. Medium conversion corn syrup ('C.S.U.', 'confectioners' glucose') differs from these only in that it lacks added sucrose and coloring. The Argentine and Mexican syrups were imported for sale in Canada as sugar substitutes. The wheat and potato syrup samples represent

TABLE I

COMPOSITION OF COMMERCIAL STARCH SYRUPS

Sample	Water, %	Protein N × 6.25, %	Ash, %	Sodium chloride, %	pH	SO_2, p.p.m.	$[\alpha]_D^{20}$ (solids)	Dextrose, %	Dextrose equivalent, % of solids
Table corn syrup	24.7	0.05	0.35	0.18	4.63	<10	142	16.2	40.8
Table corn syrup	24.1	0.12	0.24	0.11	5.51	<10	138	18.7	44.3
Corn syrup, medium conversion unmixed	19.5	0.12	0.21	0.20	5.32	290	151	17.5	40.8
Corn syrup, low conversion	18.7	0.11	0.19	0.18	5.20	<10	165	6.3	27.3
Hydrol	24.0	0.33	4.77	—	4.00	<10	—	43.5	69.3
Argentine corn syrup	20.1	0.22	0.36	0.24	4.79	<10	151	18.7	38.5
Mexican corn syrup	17.4	0.10	0.35	0.24	5.08	340	154	15.4	36.2
Potato syrup	18.5	0.07	0.28	0.23	4.30	<10	141	22.5	39.4
		(N × 5.7)							
Wheat syrup No. 1	18.6	0.10	0.25	0.22	4.58	<10	126	27.7	53.7
Wheat syrup No. 2	23.3	1.01	0.64	0.46	5.22	<10	127	22.4	51.4
Wheat syrup No. 3	23.9	0.50	0.81	0.63	4.98	<10	126	26.0	51.1
Wheat syrup No. 4	27.2	0.83	1.58	0.64	4.95	<10	—	14.6	37.0
Wheat syrup No. 5	28.7	0.65	1.51	0.68	5.00	<10	—	23.3	41.5
Wheat syrup No. 6	20.7	0.68	1.25	0.38	5.15	<10	145	23.4	39.4

wartime products that were produced in Canada to the extent of 500 to 1000 tons per month.

The water contents ranged from 17.4 to 28.7%. Samples containing over 25% water fermented during subsequent storage, whereas those containing less than 19% water were extremely viscous and difficult to handle.

The protein content of the corn and potato syrups was approximately 0.1%, whereas that of the commercial wheat syrups usually was between 0.5 and 1.0%. The low protein content shown by wheat syrup No. 1 indicates that the starch employed in its preparation was of high quality. This sample was very similar in taste and appearance to the potato and medium conversion corn syrup samples. The syrups of higher nitrogen content usually had considerable off-taste, were strongly colored, and developed turbidity and flocculent precipitates after lengthy storage periods.

According to Parow (14), potato syrup is superior to corn syrup for making confectionery because of its lower nitrogen content and correspondingly diminished tendency to develop color in the candy kettle. Some support for this is provided by the fact that commercial corn starch usually contains a higher content of nitrogeneous impurities than potato starch. The corn syrups employed by Parow in his comparisons, however, were prepared from corn starches of exceptionally high protein content (0.5% and higher). Similar heating tests applied to the present commercial products indicated lesser discoloration by the corn and potato syrups than by the wheat syrups. No difference in color development was detected between the corn and potato

syrups, however, and the same result was obtained with corn and potato syrups prepared in the laboratory.

The total ash and sodium chloride values of Table I indicate large variations in content of extraneous inorganic matter. Impurities introduced with the process water or by corrosion have deleterious effects such as storage haze (6), promotion of discoloration (6), and production of an astringent taste (2, p. 130). Most of the wheat syrups contained sufficient sodium chloride to impart a perceptibly salty taste. The high sodium chloride content of 'Hydrol' is a result of the large amount of hydrochloric acid employed in dextrose production (up to 1% hydrochloric acid on the dry weight of starch) and of the concentration of salt in the mother liquor that accompanies dextrose crystallization.

The pH of all syrups was approximately 5.0, which apparently was the neutralization level used in their production. The sulphur dioxide content of starch syrups is not subject to statutory regulation in Canada, and in two instances exceeded 250 p.p.m.

The dextrose equivalent or 'purity', true dextrose, and specific rotation values reflect different aspects of starch hydrolysis, but under standard conditions of manufacture any one of these could be used to designate degree of conversion. 'Medium purity' corn syrups showed specific rotation values of approximately 150, true dextrose contents of approximately 18%, wet basis, and dextrose equivalent values of 40 or slightly higher. The 'low purity' or brewers' body syrup had a specific rotation above 160, true dextrose contents of less than 10%, and dextrose equivalent values of 25 to 32. The high content of complex dextrins in the latter syrup rendered it densely opaque. The degree of conversion shown by the sample of Mexican corn syrup (36 D.E.) represents the borderline between 'body' and 'regular' syrups. Hydrolysis in this instance had proceeded just far enough to prevent the development of opacity. Most of the wheat syrups showed specific rotation values of 125 to 130, dextrose contents of approximately 25%, and dextrose equivalent values of 50 or higher. As these products were intended for use as sweeteners, their hydrolysis was often allowed to proceed further than in the production of 'medium' purity corn syrup.

Ammonium Chloride in Starch Syrups

Amide groups of the protein impurities in the raw starch could be liberated by hydrolysis and appear in finished syrup as ammonium chloride. Laboratory experiments provided support for this supposition and indicated that high concentrations of acid, as were being used in wheat syrup production, stimulated the production of ammonium chloride. It was also observed that addition of as little as 25 mgm. % ammonium chloride conferred a definite 'off-taste' to table corn syrups, and that 7 mgm. % ammonium chloride was detectable in tap water.

Folin's adapted method (10) gave the following ammonia values, expressed as ammonium chloride, for commercial starch syrups: table corn syrup,

9 mgm. %; unmixed corn syrup, 11.6 mgm. %; wheat syrup No. 1, 11.6 mgm. %; wheat syrup No. 2, 18.1 mgm. %; and wheat syrup No. 4, 222 mgm. %. Among these syrups, only the last, which was prepared by the acid hydrolysis of wheat flour, was found to contain sufficient ammonium chloride to exceed the concentration at which its objectionable taste is perceptible.

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References

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. Bardorf, C. F. and Ball, J. A. B. Elements of sugar refining. Chemical Publishing Company, Easton, Pa. 1925.
3. Cleland, J. E. and Fetzer, W. R. Ind. Eng. Chem., Anal. Ed. 13 : 858-860. 1941.
4. Cleland, J. E. and Fetzer, W. R. Ind. Eng. Chem., Anal. Ed. 14 : 124-127. 1942.
5. Cleland, J. E., Evans, J. W., Fauser, E. E., and Fetzer, W. R. Ind. Eng. Chem., Anal. Ed. 16 : 161-165. 1944.
6. Clendenning, K. A. and Wright, D. E. Can. J. Research, (F). 1948. In press.
7. Fauser, E. E., Cleland, J. E., Evans, J. W., and Fetzer, W. R. Ind. Eng. Chem., Anal. Ed. 15 : 193-200. 1943.
8. Fetzer, W. R. and Evans, J. W. Ind. Eng. Chem. 28 : 885-888. 1936.
9. Fetzer, W. R., Evans, J. W., and Longenecker, J. B. Ind. Eng. Chem., Anal. Ed. 5 : 81-84. 1933.
10. Folin, O. Z. physiol. Chem. 37 : 161-176. 1902.
11. Jensen, H. R. Analyst 53 : 133-135. 1928.
12. Kerr, R. W. Chemistry and industry of starch. Academic Press, Inc., New York. 1944.
13. Kröner, W., Reischel, W., and Höppner, W. Z. f. anal. Chem. 122 : 321-334. 1941
14. Parow, E. Z. f. spiritusind. 45 : 229. 1922.
15. Sair, L. and Fetzer, W. R. Cereal Chem. 19 (Suppl.) 633-655. 1942.
16. Sair, L. and Fetzer, W. R. Cereal Chem. 19 (Suppl.) 655-668. 1942.
17. Sair, L. and Fetzer, W. R. Cereal Chem. 19 (Suppl.) 714-715. 1942.
18. Schultz, A. S., Fisher, R. A., Atkin, L. and Frey, C. N. Ind. Eng. Chem., Anal. Ed. 15 : 496-498. 1943.
19. Shapiro, E., and Proferansova, M. N. Z. Wirtschaftsgruppe Zuckerind. 85 : 196-199. 1935.
20. Sichert, K. and Bleyer, B. Z. f. anal. Chem. 107 : 328-338. 1936.
21. Steinhoff, G. Z. f. spiritusind. 56 : 64-65. 1933.
22. Steinhoff, G. Z. f. spiritusind. 56 : 73-74. 1933.
23. Wayne, T. B. Ind. Eng. Chem. 18 : 847-854. 1926.
24. Weintraub, R. L. and Price, L. Smithsonian Misc. Collection 104, No. 10. 1945.
25. Zerban, F. W. and Sattler, L. Ind. Eng. Chem., Anal. Ed. 10 : 669-674. 1938.

POLARIMETRIC DETERMINATION OF STARCH IN CEREAL PRODUCTS

V. THE PARTICLE SIZE AND EXTRACTION TIME REQUIREMENTS[1]

By K. A. Clendenning[2]

Abstract

Grinding wheat to pass a 40 mesh screen is adequate for polarimetric starch analyses. Finer grinding does not influence the precision, apparent starch content, or extraction time requirement appreciably. The pentosan content of calcium chloride extracts is increased by fine grinding and by prolonged extraction and is decreased by preliminary extraction with dilute alkali. The wheat products employed in this study were subjected to complete analysis by conventional methods (ash, fat, protein, sugar, pentosan, polyuronide, fiber). Starch contents calculated by difference were in close agreement with direct determinations on patent flour, and showed differences of 1.3 to 2.6% on whole wheat. Apparent fat contents, determined with ethyl ether or petroleum ether, were doubled by preliminary acid digestion. Wheat is shown to have an appreciable polyuronide content, which is located in the integuments.

Introduction

Preparatory grinding to an impalpable flour has been prescribed almost invariably in polarimetric procedures for starch in cereal grains (9, 11, 12, 16, 17). The directions of Ewers (11) and Hopkins (12) specify samples that pass 125 and 100 mesh screens, respectively. This requirement is usually time-consuming in routine analyses, and except for wheat gluten (4), is not supported by published data. Earle and Milner (8) recommend grinding to pass the 0.5 mm. Wiley screen in the analysis of wheat and corn. Lampitt *et al.* (15) have shown that lengthy ball-milling greatly increases the solubility of starch in cold water. If the samples are extracted with cold water or dilute alkali prior to starch dispersion and polarization (8), starch losses then become possible as a result of thorough grinding.

The present paper provides information on the particle size and extraction time requirements in calcium chloride polarimetric procedures for starch in whole wheat and wheat flour. The samples were subjected to complete analysis by conventional methods to provide approximate values for starch by difference. Experiments also are reported on the effects of sample size on starch recovery, and of particle size and extraction time on pentosan solubilization.

Materials and Methods

Hard Red Spring wheat samples were ground in a Wiley mill equipped with bottom screens having 1 mm. and 2 mm. circular openings. Subsamples were

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ball-milled, and the accompanying increase in ash content (0.1 to 0.3%) was allowed for in the calculations. The wide range of particle sizes represented by the samples used in this study is shown in Table I.

TABLE I

SIEVE ANALYSES OF WHOLE WHEAT AND GRANULAR FLOURS, %

Screen mesh	Whole wheat 1			Whole wheat 2		Granular flour	
	Wiley-milled, 2 mm. screen	Wiley-milled, 1 mm. screen	Ball-milled	Wiley-milled, 1 mm. screen	Ball-milled	As received	Ball-milled
Retained on 20	36.3	Nil	Nil	Nil	Nil	Nil	Nil
Retained on 40	47.6	51.7	1.4	69.7	1.6	26.3	Nil
Retained on 60	5.8	23.6	5.2	12.9	6.6	35.8	Nil
Retained on 80	2.1	4.8	4.2	3.7	3.9	13.1	0.1
Retained on 100	1.9	1.9	1.1	2.1	2.3	11.1	0.3
Passed 100	6.3	16.0	88.1	11.6	85.6	13.7	99.6

Ash and crude fiber contents were determined by the conventional A.O.A.C. methods (2). In calculating protein contents from Kjeldahl nitrogen values, a factor of 5.7 was used for wheat flours and of 5.83 for whole wheat as recommended by Jones (14). Total sugar was estimated by the A.A.C.C. ferricyanide method (1). Moisture was determined by drying *in vacuo* at 100° C. for 20 to 30 hr. Values obtained by this method on starches, flour, and whole wheat agreed within 0.1% with those obtained by 60 hr. distillation with toluol under the conditions prescribed by Sair and Fetzer (18). The A.O.A.C. acid hydrolysis–ether extraction method (2, p. 240), which was selected for the determination of crude fat contents, gave values approximately twice as high as those observed with 24-hr. ethyl ether and petroleum ether extraction treatments of various wheat fractions (Table II).

TABLE II

A COMPARISON OF METHODS FOR THE DETERMINATION OF CRUDE FAT IN WHEAT PRODUCTS, %

	Ethyl ether*	Petroleum ether*	Acid hydrolysis –ether
Wheat starch	0.28	Nil	0.48
Wheat flour	0.71	0.75	1.60
Whole wheat flour	2.08	1.80	3.00
Gluten (100 mesh)	0.63	0.43	5.00

* *By 24-hr. Soxhlet extraction.*

In determining pentosan contents, 5-gm. samples were distilled with 12% hydrochloric acid at the usual rate of 3 ml. per min. until a negative test for furfural was obtained with aniline acetate. The distillates were redistilled at the same rate in the presence of 20% sodium chloride (19). A correction

of 3% was applied for furfural destruction in the second distillation. Furfural was determined at 0° C. by the Hughes–Acree method (13). In accordance with Krober's formula (2, p. 413), the yield of furfural in the first distillation was assumed to be 83% of the theoretical, or 58.5% of the pentosan content.

Polyuronide content was estimated by decarboxylation of 5- to 10-gm. samples in accordance with the directions of Dickson *et al.* (7). The Truog absorption tower assembly was constructed entirely of glass, 34/45 ST joints being substituted for the troublesome rubber connections on the column. Campbell *et al.* (3) have recommended the use of an aniline trap as a means of excluding furfural from the absorption tower. Tests with freshly distilled furfural showed it to have a slight neutralizing action on barium hydroxide, 1 gm. of furfural being equivalent to 1.94 ml. *N*/10 hydrochloric acid or 4.3 mgm. carbon dioxide. As the samples with which we were concerned produced 200 mgm. or less of furfural, its exclusion (3) appeared to be an unnecessary refinement. In agreement with Whistler *et al.* (20), production of 2.64 mgm. carbon dioxide per gram of pure glucose, or per gram of starch expressed as glucose, was observed with the usual five hour refluxing period. A correction based on the measured starch and sugar contents of the samples was accordingly made for this source of carbon dioxide in the polyuronide determinations. The corresponding yield of carbon dioxide from gliadin and gluten samples was 0.85 mgm. carbon dioxide per gram, for which corrections were not introduced.

In testing the effects of particle size on starch recovery and reproducibility, the extraction was conducted under the usual conditions (6) for periods of 15, 30, and 45 min. Judging from previous observations on pure starches (5), the effects of lengthened extraction treatments on the specific rotation of starch may be neglected. Protein precipitation and filtration were conducted as previously directed (6). All filtrations were rapid and the filtrates without exception permitted accurate polarimetric observations on 2-dm. tubes.

Results

Marked improvement in starch recovery and reproducibility (Table III) as a result of further grinding or prolonged extraction was observed only on the coarsest sample (Whole Wheat 1, 2 mm.). It should be noted that 36% of this sample would not pass a 20 mesh screen (Table I). Starch recovery from all samples ground in the Wiley mill to pass either a 1 mm. or 40 mesh screen was not increased by ball-milling to pass a 100 mesh screen or by extending the extraction to 30 or 45 min. (Table III). Since more than 50% of the 1 mm. samples would not pass a 40 mesh screen, grinding to the latter mesh size is adequate by a considerable margin. Agreement between duplicates on all but the coarsest sample was within 0.3% starch. The apparent starch content of the 40 mesh samples also was not changed (within 0.3%) by reducing the sample size from 2.5 to 1.5 or 0.5 gm.

Corresponding studies of the wheat gluten particle size requirement (4) indicated the necessity of extending the extraction to 40 min. for maximum

TABLE III

EFFECTS OF PARTICLE SIZE AND EXTRACTION TIME ON STARCH RECOVERY FROM WHOLE WHEAT AND GRANULAR FLOURS

Sample	Apparent starch content,* %		
	Extracted 15 min.	Extracted 30 min.	Extracted 45 min.
Whole Wheat I, 2 mm. screen	57.84	60.42	60.42
	59.09	60.30	60.52
Whole Wheat I, 1 mm. screen	62.09	62.42	62.09
	62.32	62.32	62.32
Whole Wheat I, 40 mesh	62.16	61.93	61.60
	62.26	62.04	61.60
Whole Wheat I, ball-milled	61.66	61.46	61.34
	61.66	61.46	61.46
Whole Wheat II, 1 mm. screen	58.74	58.64	58.17
	58.74	58.74	58.17
Whole Wheat II, 40 mesh	59.03	59.03	58.60
	58.93	58.82	58.47
Whole Wheat II, ball-milled	58.81	58.81	58.04
	58.93	58.70	58.04
Granular flour as received	76.82	76.82	76.82
	77.14	76.82	76.82
Granular flour ball-milled	76.84	76.41	76.18
	76.84	76.61	76.28

* *Data are expressed on a basis of total solids, corrected for ash increments in milling.*

starch recovery from 140 mesh samples. Evidently the wet separation of gluten and subsequent drum-drying treatments render it highly impervious to the starch solvent. Meeting the particle size requirement in this instance is simplified by the uniformity in composition of the different-sized gluten particles after coarse grinding. Analysis of the 'flour' fraction obtained by sieving Wiley-milled gluten samples in our experience has given the same result as when the entire sample is ball-milled to the required particle size.

It has been shown (6) that solubilization of bran hemicelluloses by boiling calcium chloride solution is promoted by high extraction temperatures and salt concentrations. The data of Table IV indicate that solubilization of wheat pentosans [*levo*-rotatory (6)] is also increased by reduced particle size and prolonged extraction. These differences are not sufficiently large, however, to influence polarimetric starch data. Similar experiments indicated that the pentosan content of the calcium chloride filtrate is reduced by 25% when 40 mesh whole wheat is subjected to preliminary extraction with dilute alkali as recommended by Earle and Milner (8).

Eva and Rankin (10) have previously provided extensive comparative data for starch analyses of whole wheat by five direct methods. Table V provides

data for polarimetric starch determinations by two methods, and for starch 'by difference' based on conventional methods for nonstarch constituents. The latter values should be regarded as approximations because of the doubtful accuracy of the methods employed in their calculation. Close agreement

TABLE IV

EFFECTS OF PARTICLE SIZE AND EXTRACTION TIME ON PENTOSAN SOLUBILIZATION

(Milligrams pentosan dissolved per gram wheat solids)

Extraction time, min.	40 mesh	100 mesh
15	22.7	25.4
45	30.2	30.9

TABLE V

COMPOSITION OF WHEAT FLOURS, PER CENT, SOLIDS BASIS

	Whole Wheat I	Whole Wheat II	Granular flour	Patent flour
Ash	1.92	1.87	0.76	0.48
Fat (by acid hydrolysis)	3.82	4.42	1.45	1.58
Protein	15.09	19.52	13.27	15.04
Total sugar	4.10	4.00	2.34	2.18
Pentosan	6.64	7.25	2.91	1.93
Polyuronide	0.78	0.86	Trace	Trace
Crude fiber	2.63	2.78	0.71	0.18
Starch by difference	64.82	60.30	78.56	78.61
Polarimetric starch (alcohol extraction)	62.16	59.00	77.25	78.5
Polarimetric starch (stannic chloride precipitation)	62.20	58.96	76.84	78.2

is shown between the starch 'by difference' and 'by analysis' data for patent flour. Discrepancies of 1.3 to 2.6% are shown by the whole wheat data, and of 1.25 to 1.7% by the granular flour analyses.

Polyuronide determinations on wheat starch, patent flour, and gluten, corrected for carbon dioxide production by glucose and pure gliadin in terms of total carbohydrate and protein, indicated negligible contents of uronic acid residues. Corresponding analyses of whole wheat and 100 mesh wheat bran indicated contents of 0.8 and 3.9% respectively. In contrast to pentosans, which are found in all parts of the wheat kernel, the polyuronide fractions apparently are restricted to the integuments.

Acknowledgment

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References

1. American Association of Cereal Chemists. Cereal laboratory methods. 4th ed. A.A.C.C., Lincoln, Nebraska. 1941.
2. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 6th ed. A.O.A.C., Washington, D.C. 1945.
3. Campbell, W. G., Hirst, E. L., and Young, G. T. Nature 142 : 912-913. 1938.
4. Clendenning, K. A. Can. J. Research, C, 20 : 403-410. 1942.
5. Clendenning, K. A. Can. J. Research, B, 23 : 113-130. 1945.
6. Clendenning, K. A. Can. J. Research, B, 23 : 239-259. 1945.
7. Dickson, A. D., Otterson, H., and Link, K. P. J. Am. Chem. Soc. 52 : 775-779. 1930.
8. Earle, F. R. and Milner, R. T. Cereal Chem. 21 : 567-573. 1944.
9. Effront, J. La Bière 4 : 145-147. 1896.
10. Eva, W. J. and Rankin, E. E. Can. J. Research, B, 23 : 260-268. 1945.
11. Ewers, E. Z. öffentl. Chem. 15 : 8-14. 1909.
12. Hopkins, C. Y. Can. J. Research, 11 : 751-758. 1934.
13. Hughes, E. E. and Acree, S. F. Ind. Eng. Chem., Anal. Ed. 6 : 123-124. 1934.
14. Jones, D. B. Cereal Chem. 3 : 194-198. 1926.
15. Lampitt, L. H., Fuller, C. H. F., and Goldenberg, N. J. Soc. Chem. Ind. 60 : 301-305. 1941.
16. Lintner, C. J. Z. Nahr. Genussm. 14 : 205-208. 1907.
17. Mannich, C., and Lenz, K. Z. Nahr. Genussm. 40 : 1-11. 1920.
18. Sair, L., and Fetzer, W. R. Cereal Chem. 19(suppl.) 714-718. 1942.
19. Schmidt-Nielsen, S., and Hammer, L. Kgl. Norske Videnskab. Selskabs, Forh. 5 : 84-87. 1932.
20. Whistler, R. L., Martin, A. R., and Harris, M. J. Research Natl. Bur. Standards, 24 : 13-23. 1940.

THIAMINE AND RIBOFLAVIN CONTENT OF WHEAT, BARLEY, AND OATS GROWN IN DIFFERENT SOIL ZONES IN ALBERTA[1]

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Abstract

A study was made of the thiamine and riboflavin content of Marquis wheat, Newal barley, and Victory oats grown in Alberta in the years 1944 and 1945 under widely different conditions of soil and climate. The mean thiamine content of Victory oats, 5.6 ± 1.5 μgm. per gm., was found to be significantly higher than that of Marquis wheat, 4.4 ± 1.2, or of Newal barley, 4.6 ± 1.0 μgm. per gm. A positive correlation between protein and thiamine content was demonstrated in wheat and oats but not in barley. In all three grains the mean thiamine content of samples grown in the brown soil zone was significantly higher than that of samples grown in the gray soil zone. The mean values obtained for riboflavin were 1.34 ± 0.23, 1.25 ± 0.25, and 1.27 ± 0.18 μgm. per gm. in wheat, barley, and oats, respectively. No significant correlation between protein and riboflavin was found in any of the three cereals. The mean riboflavin content of samples of wheat, barley, and oats grown on gray soils was slightly lower than that of samples grown on black or brown soils.

Introduction

Owing to the fact that a relatively high proportion of the calorie intake of both humans and farm livestock is made up of cereal grains or their products, the questions of vitamin levels and factors which may influence these levels in grains are of considerable interest. The results of several investigations (3, 5, 8, 9, 10, 16) indicate that, in wheat, thiamine levels may be related to a number of variables including type and variety, protein and ash content, kernel and bushel weight, and the environment in which the grain is grown. The factors which may influence or be related to thiamine levels have been less thoroughly studied in barley and oats than in wheat.

Data regarding the riboflavin content of Canadian grains are meager. The results of studies conducted in the United States (2, 3) show that the riboflavin content of cereal grains is lower and less variable than is the thiamine content, and suggest that such factors as environment, variety, and protein content may be less important in relation to riboflavin than to thiamine accumulation.

The objectives of the present investigation were:

(1) To obtain data on the relative thiamine and riboflavin content of wheat, barley, and oats grown in Alberta.

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(2) To study the correlation between protein and vitamin content in each of these cereal grains.

(3) To determine whether vitamin content could be related to the type of soil on which the grains were grown and whether such relation between thiamine or riboflavin content and soil is the same in all three grains.

Experimental

Assay Material

The grains studied were Marquis wheat, Newal barley, and Victory oats grown in the years 1944 and 1945. Samples were chosen to cover a wide

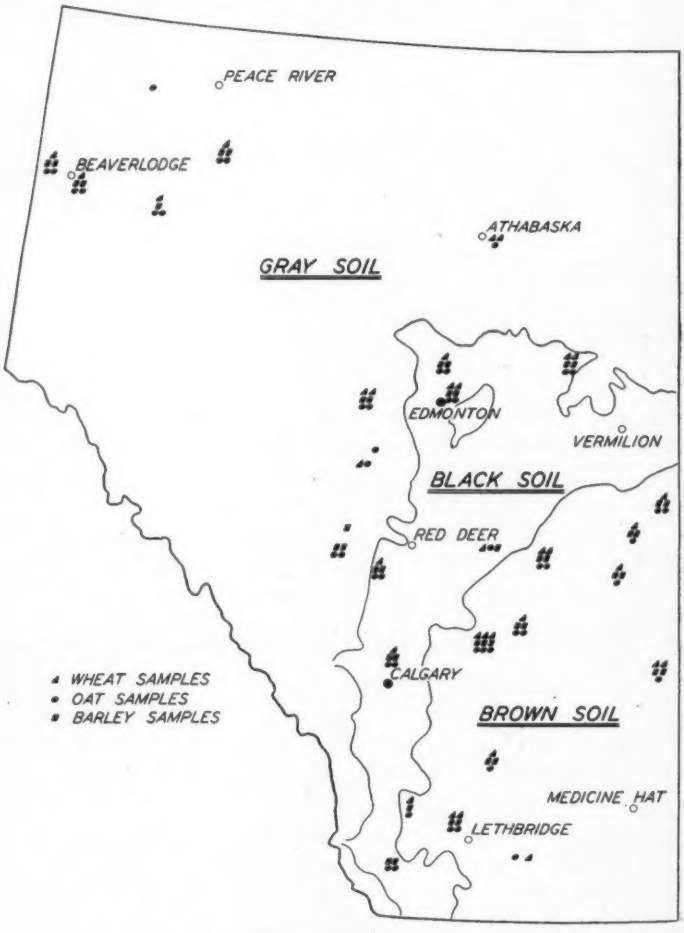

FIG. 1. *Locations in which samples were grown.*

range in protein content and to represent grains grown under a variety of soil and climatic conditions. They were obtained from University of Alberta test plots and from Dominion Illustration and Experimental Stations located in representative soil zones throughout the province of Alberta. The locations from which grain samples were secured, together with an outline of the major soil zones in Alberta, are given in Fig. 1. Additional information regarding the location, climate, and fertility of the soil zones of the province is given in "Soil Zones of Alberta" (14). An attempt was made to obtain an equal number of samples of each grain from each location, but as is evident from the distribution map (Fig. 1) this was not possible in all cases. It should also be noted that in Fig. 1 and in the text, gray and gray transition zones are grouped as 'gray,' black and shallow black as 'black,' and brown and dark brown as 'brown' soil zones.

Assay Methods

Protein.—Nitrogen was determined using the Kjeldahl method with mercuric oxide as catalyst. Protein in wheat is reported as nitrogen × 5.7 and in oats and barley as nitrogen × 6.25. All protein and vitamin assay results are expressed on the basis of 13.5% moisture.

Thiamine.—Thiamine assays were conducted on finely ground samples of grain by the yeast fermentation method of Schultz, Atkin, and Frey (12). Representative values obtained by this method as compared to those obtained by a modification of the Hennessy (6) thiochrome method are given in Table I. The values shown are averages of the results of a minimum of four assays done in duplicate. Although some variation is apparent in the thiamine values of individual samples as determined by the two methods, the means for wheat and barley do not suggest that there is any significant difference

TABLE I

FERMENTATION AND THIOCHROME THIAMINE ASSAY VALUES FOR CEREAL GRAINS

Grain	Sample	Protein, %	Fermentation, μgm./gm.	Thiochrome, μgm./gm.
Wheat	1	19.7	6.4	5.6
	2	13.7	4.9	4.6
	3	9.2	3.6	4.1
			Mean 5.0	4.7
Barley	1	17.1	7.4	7.6
	2	13.0	4.3	4.0
	3	9.4	4.1	4.3
			Mean 5.3	5.3
Oats	1	16.3	7.6	8.4
	2	12.3	5.4	6.2
	3	9.6	4.2	5.0
			Mean 5.7	6.5

between fermentation and thiochrome assay values for these two cereals. Thiamine levels in oats, as measured by the thiochrome method, were about 15% higher than those obtained by the fermentation method.

Riboflavin.—Riboflavin assays were done by a modification of the method of Snell and Strong (13) using *Lactobacillus casei-ϵ* as the test organism. Samples were prepared by autoclaving in 0.1 *N* hydrochloric acid for 20 min. at 15 lb. pressure, followed by adjustment to pH 4.5, filtration, and extraction with ether as recommended by Strong and Carpenter (15). It was found necessary to add 1 ml. of a saturated solution of dibasic potassium phosphate to the extracts after ether treatment in order to precipitate excess calcium. After incubation for 72 hr., the contents of the assay tubes were rinsed into 100 ml. beakers and titrated with 0.1 *N* sodium hydroxide to pH 6.8, as measured by a potentiometer fitted with outside electrodes. As is shown in Table II, results obtained by this method were in good agreement with those obtained by the fluorometric method described by Andrews (1).

TABLE II

COMPARISON OF MICROBIOLOGICAL AND FLUOROMETRIC RIBOFLAVIN ASSAY RESULTS FOR WHEAT, BARLEY, AND OATS

Grain	Sample	Protein, %	Method	
			Microbiological, μgm./gm.	Fluorometric, μgm./gm.
Wheat	1	19.7	1.32	1.25
	2	13.7	1.22	1.16
	3	9.2	1.20	1.05
			Mean 1.25	1.15
Barley	1	17.1	1.23	1.24
	2	13.0	1.50	1.33
	3	9.4	1.28	1.23
			Mean 1.33	1.27
Oats	1	16.3	1.54	1.58
	2	12.3	1.23	1.28
	3	9.6	1.36	1.26
			Mean 1.37	1.37

Results

A total of 31 samples of Marquis wheat, 43 of Newal barley, and 50 of Victory oats were assayed for thiamine. A few additional samples were assayed for riboflavin so that the totals for numbers of samples assayed for this vitamin are 37, 47, and 50 for wheat, barley, and oats, respectively. The results are summarized in Table III.

The mean of 4.4 μgm. per gm. for the thiamine content of Marquis wheat is in good agreement with the value of 4.3 μgm. per gm. (653 I.U. per lb.)

TABLE III

THIAMINE AND RIBOFLAVIN CONTENT OF GRAINS GROUPED ACCORDING TO YEAR

Year	No. of samples	Mean protein, %	Vitamin, μgm./gm.	
			Mean	S.d.
Thiamine				
Marquis wheat				
1944	17	12.8	3.82	0.74
1945	14	14.4	5.11	0.98
	31	13.5	4.40	1.20
Newal barley				
1944	22	12.3	4.59	1.15
1945	21	13.9	4.60	0.90
	43	13.1	4.60	1.02
Victory oats				
1944	26	10.9	5.35	1.41
1945	24	12.6	5.84	1.61
	50	11.7	5.58	1.51
Riboflavin				
Marquis wheat				
1944	20	12.9	1.25	0.15
1945	14	14.4	1.45	0.27
	34	13.5	1.34	0.23
Newal barley				
1944	26	12.4	1.25	0.28
1945	21	13.9	1.24	0.22
	47	13.1	1.25	0.25
Victory oats				
1944	26	10.9	1.21	0.09
1945	24	12.6	1.35	0.22
	50	11.7	1.27	0.18

reported by Whiteside and Jackson (16) as the mean for a large number of samples of wheat grown in Western Canada. The mean thiamine values obtained for Newal barley and Victory oats are somewhat lower than others that have been reported for barley and oats grown in the United States (4, 10, 11). The riboflavin assay results are similar to those obtained by Andrews, Boyd, and Terry (2), who report means of 1.32 μgm. per gm. for riboflavin in Marquis wheat grown at four different stations in Minnesota, and means of 1.21 and 1.30 μgm. per gm. for riboflavin in six varieties of barley and five varieties of oats, respectively.

Application of the *t* test to the present data showed that the difference between the mean thiamine content of the oat samples and that of wheat or

barley is significant beyond the 1% point. The differences between the three cereals in riboflavin content are not statistically significant.

The relation between protein and thiamine content was studied by grouping all the thiamine assay values for each of the three grains without regard to year or origin. It is shown in Table IV that a highly significant positive correlation was found to exist between protein and thiamine in wheat and oats but not in barley. Scatter diagrams for each of the three grains are

TABLE IV

CORRELATION COEFFICIENTS BETWEEN PROTEIN AND THIAMINE IN WHEAT, BARLEY, AND OATS

Cereal	D.f.	r	5% pt.	1% pt.
Marquis wheat	29	0.513**	0.356	0.456
Newal barley	41	0.266	0.301	0.389
Victory oats	48	0.652**	0.279	0.361

** *Significant beyond 1% point.*

given in Fig. 2. A similar analysis of the riboflavin results indicated that there was no significant correlation between protein and riboflavin in any of the three cereals.

In an attempt to assess the effect of soil zone on the thiamine and riboflavin content of wheat, barley, and oats, the data were grouped and analyzed according to soil zones and years, as shown in Tables V and VI. Reference to Table V shows that the mean thiamine content of all three grains was lowest for samples grown on gray soils, intermediate for those from black soils, and highest for those grown on brown soils. The existence of this soil zone–thiamine relation in all three grains is interesting, since the barleys assayed differed from the wheat and oats in that they failed to demonstrate a significant correlation between protein and thiamine. This suggests that soil zone may have more effect on thiamine accumulation than has protein content as such.

The results of analysis of variance (Table VI) indicate that, in general, the soil zone–thiamine relations suggested by the data in Table V are statistically significant. However, the variability in the thiamine content of individual samples within a given zone was so great that the accuracy of estimates of thiamine levels in grains based on soil zone–thiamine correlations is not likely to be high. Nonetheless, the fact that further analysis of the data as summarized in Table VII shows that for all three grains the mean thiamine content of the samples grown on brown soils was significantly higher than that of samples grown on gray soils suggests that soil zone–thiamine relations may be of practical significance when grains grown on widely different types of soil and under markedly different climatic conditions are under consideration. The differences between mean thiamine levels in grains grown under some-

what more similar conditions were not so marked. Thus, differences between the mean thiamine content of samples grown on black soil and those grown on gray soil were highly significant only in the case of oats, while for the brown-black soil zone comparison highly significant differences existed only in wheat.

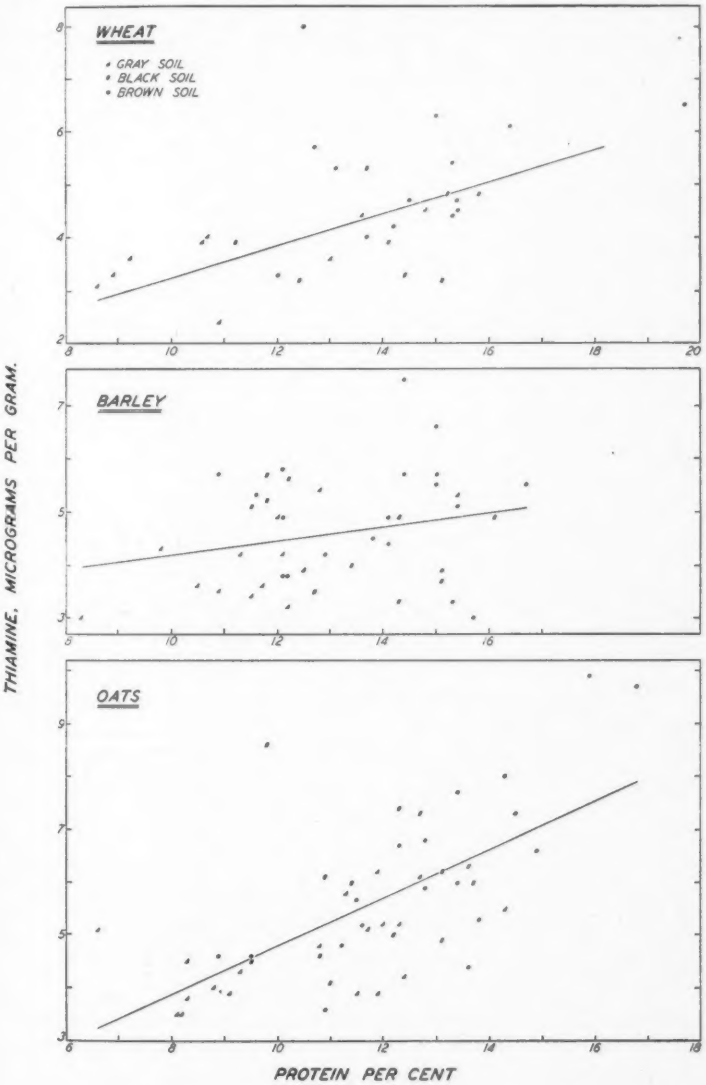

FIG. 2. *Relation between thiamine and protein content of grains.*

TABLE V

THIAMINE AND RIBOFLAVIN CONTENT OF GRAINS GROUPED ACCORDING TO SOIL ZONES AND YEARS

Soil zone	Marquis wheat				Newal barley				Victory oats			
	No. of samples	Mean protein, %	Vitamin, μgm./gm.		No. of samples	Mean protein, %	Vitamin, μgm./gm.		No. of samples	Mean protein, %	Vitamin, μgm./gm.	
			Mean	S.d.			Mean	S.d.			Mean	S.d.
Thiamine												
Gray and gray transition												
1944	7	10.9	3.50	0.66	6	11.0	3.60	0.67	9	9.4	4.46	0.76
1945	2	12.0	4.10	—	7	12.1	4.40	0.77	10	11.5	4.84	0.94
	9	11.1	3.6	0.66	13	11.6	4.04	0.77	19	10.5	4.65	0.86
Black and shallow black												
1944	5	14.1	3.68	0.69	7	12.7	4.76	0.97	9	11.7	5.52	0.98
1945	3	14.5	4.67	0.71	3	14.9	4.15	0.63	4	12.2	7.05	1.30
	8	14.2	4.05	0.83	10	13.3	4.59	0.89	13	11.9	5.99	1.25
Brown and dark brown												
1944	5	14.2	4.36	0.72	9	12.8	5.12	1.19	8	11.8	6.15	1.88
1945	9	14.8	5.50	1.41	11	14.8	4.85	1.04	10	13.8	6.36	1.76
	14	14.7	5.09	1.30	20	13.9	4.97	1.09	18	12.9	6.27	1.76
Riboflavin												
Gray and gray transition												
1944	7	10.9	1.18	0.16	6	11.0	1.06	0.24	9	9.4	1.16	0.10
1945	2	12.0	1.28	—	7	12.1	1.28	0.27	10	11.5	1.22	0.24
	9	11.1	1.20	0.15	13	11.6	1.18	0.27	19	10.5	1.19	0.11
Black and shallow black												
1944	6	13.8	1.25	0.23	10	13.0	1.31	0.26	9	11.7	1.20	0.09
1945	3	14.5	1.37	0.62	3	14.9	1.55	0.23	4	12.2	1.54	0.13
	9	14.1	1.40	0.39	13	13.5	1.36	0.27	13	11.9	1.31	0.19
Brown and dark brown												
1944	7	14.3	1.33	0.13	10	12.5	1.30	0.30	8	11.8	1.26	0.09
1945	9	14.8	1.41	0.13	11	14.8	1.14	0.05	10	13.8	1.39	0.17
	16	14.6	1.38	0.13	21	13.7	1.21	0.22	18	12.9	1.33	0.15

Thiamine surveys done by Johannson and Rich (9) on commercial Western Canadian wheats grown in 1940 and by Hoffer, Alcock, and Geddes (7) on the 1941 crop failed to demonstrate a significant effect of soil zone on thiamine content although the results obtained in the latter survey did suggest that a relation between thiamine and soil zone might exist inasmuch as it was found

TABLE VI

ANALYSIS OF VARIANCE FOR THIAMINE AND RIBOFLAVIN

Variance due to	Wheat		Barley		Oats	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Thiamine						
Soil zone	2	6.42**	2	3.42**	2	13.43**
Year	1	12.91**	1	0.00	1	3.06
Error	27	0.650	39	0.946	46	1.775
Total	30		42		49	
Riboflavin						
Soil zone	2	0.166*	2	0.127	2	0.98*
Year	1	0.220*	1	0.00	1	0.248**
Error	30	0.044	43	0.062	46	0.026
Total	33		46		49	

* *Significant beyond the 5% point.*
** " " " *1%* "

TABLE VII

t VALUES DERIVED TO TEST THE SIGNIFICANCE OF DIFFERENCES IN THE MEAN THIAMINE CONTENT OF SAMPLES GROWN ON DIFFERENT TYPES OF SOIL

——	Wheat		Barley		Oats	
	No. of samples	*t*	No. of samples	*t*	No. of samples	*t*
Between soil zones:						
Black-gray	17	1.77	23	2.24*	32	5.11**
Brown-gray	23	5.40**	33	3.96**	37	5.66**
Brown-black	22	3.26**	30	1.45	31	0.74

* *Significant beyond the 5% point.*
** " " " *1%* "

that wheat samples from the dark brown soil zone of Saskatchewan were significantly higher in thiamine than those from the other soil zones.

With reference to the data in Table VI for variance due to year, it must be pointed out that the significance of these data is impaired by the fact that only about 30% of the wheat samples and 50% of the barley and oat samples were grown in the same locations in both years. With this limitation in mind, the results indicate that the mean thiamine content of the oat and barley samples grown in 1944 was essentially the same as that of those grown

in 1945. On the other hand, the wheat samples from the 1945 crop were significantly richer in thiamine than those grown in 1944. It seems unlikely that the higher level of thiamine in the 1945 wheat can be attributed to the higher protein content of these samples, since the three grains showed essentially the same seasonal difference in protein levels.

With regard to the results for riboflavin, it would appear from the data in Tables V and VI that the probability of the existence of significant differences in vitamin content of grain attributable to soil zone is less for riboflavin than for thiamine. As has been mentioned, the significance of the data for the effect of year is limited but it is interesting to note that for all three soil zones the riboflavin content of the oat samples grown in 1945 was appreciably greater than that of those grown in the preceding year.

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References

1. ANDREWS, J. S. Cereal Chem. 20 : 613-625. 1943.
2. ANDREWS, J. S., BOYD, H. M., and TERRY, D. E. Cereal Chem. 19 : 55-64. 1942.
3. CONNER, R. T. and STRAUB, G. J. Cereal Chem. 18 : 671-677. 1941.
4. DAVIS, C. F., LAUFER, S., and SALETAN, L. Cereal Chem. 20 : 109-113. 1943.
5. DOWNS, D. E. and CATHCART, W. H. Cereal Chem. 18 : 796-801. 1941.
6. HENNESSY, D. J. News Letter, A.A.C.C. No. 55, 25-29. 1942.
7. HOFFER, A., ALCOCK, A. W., and GEDDES, W. F. Cereal Chem. 21 : 210-222. 1944.
8. JACKSON, S. H. and WHITESIDE, A. G. O. Sci. Agr. 22 : 366-377. 1942.
9. JOHANNSON, H. and RICH, C. E. Cereal Chem. 19 : 308-313. 1942.
10. NORDGREN, R. and ANDREWS, J. S. Cereal Chem. 18 : 802-811. 1941.
11. SCHULTZ, A. S., ATKIN, L., and FREY, C. N. Cereal Chem. 18 : 106-113. 1941.
12. SCHULTZ, A. S., ATKIN, L., and FREY, C. N. Ind. Eng. Chem., Anal. Ed. 14 : 35-39. 1942.
13. SNELL, E. E. and STRONG, F. M. Ind. Eng. Chem., Anal. Ed. 11 : 346-350. 1939.
14. SOIL ZONES OF ALBERTA. 1945. A descriptive map prepared by W. Odynsky and distributed by the Department of Extension, University of Alberta, Edmonton, Alberta.
15. STRONG, F. M. and CARPENTER, L. E. Ind. Eng. Chem., Anal. Ed. 14 : 909-913. 1942.
16. WHITESIDE, A. G. O. and JACKSON, S. H. Cereal Chem. 20 : 542-551. 1943.

THE NIACIN CONTENT OF WHEAT, BARLEY, AND OATS GROWN IN DIFFERENT SOIL ZONES IN ALBERTA[1]

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Abstract

Microbiological niacin assays were done on 34 samples of Marquis wheat, 48 of Newal barley, and 50 of Victory oats grown on gray, black, and brown soils in the years 1944 and 1945. Mean values of 53.6, 70.4, and 10.3 μgm. of niacin per gram were obtained for wheat, barley, and oats respectively. Although these results suggest that Newal barley may contain more niacin than does Marquis wheat, the results of assays conducted by a chemical method indicate that there is little difference in the mean niacin content of these two grains. Niacin levels were found to be highly variable in all three grains. Niacin and protein levels were found to be positively correlated in wheat and oats while in barley a tendency towards an inverse relation between niacin and protein was observed. No evidence was obtained to indicate that the type of soil on which grains are grown has any marked effect on the accumulation of niacin. The wheat and oat samples from the 1945 crop were richer in both niacin and protein than those from the 1944 crop, while barley samples from the 1945 crop, although richer in protein than those grown the preceding year, contained essentially the same amount of niacin.

Introduction

Recognition of the importance of niacin in swine and poultry nutrition in addition to its established role in the prevention and cure of pellagra has stimulated interest in the distribution of this vitamin in cereal grains used in livestock feeds. Hale, Davis, and Baldwin (6) have reported ranges of 60 to 96, 60 to 81, and 18 to 21 μgm. per gm. in wheat, barley, and oats respectively, while Davis, Laufer and Saletan (4) list values of 49.3 to 66.1 and 69.0 to 98.1 μgm. per gm. for samples of wheat and barley of different origin and variety. Brown, Thomas, and Bina (3) have stressed the importance of existing discrepancies in niacin assay values associated with variations in methods of preparation and assay of different materials. The present study was made to:— (1) obtain information on the relative niacin content of wheat, barley, and oats grown under widely different conditions of soil and climate in the province of Alberta, and (2) study possible correlations between protein and niacin content and between soil type and niacin levels in each of these cereal grains.

Experimental

Assay Material

The grains studied were Marquis wheat, Newal barley, and Victory oats. The samples used were the same as those described by McElroy, Kastelic, and McCalla (8).

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Assay Methods

Protein

Nitrogen was determined by the Kjeldahl method with mercuric oxide as catalyst. Protein in wheat is reported as nitrogen × 5.7 and in oats and barley as nitrogen × 6.25. All protein and vitamin assay results are expressed on the basis of 13.5% moisture.

Niacin

In preliminary studies, niacin assay values obtained by the chemical method of Melnick, Oser, and Siegel (9) and by the microbiological procedure of Krehl, Strong, and Elvehjem (7) were compared. In Table I it is shown that the mean results obtained for wheat and oats by the two methods are in good agreement, while for barley the mean microbiological values are approximately 15% higher than those obtained by the chemical method.

TABLE I

CHEMICAL AND MICROBIOLOGICAL NIACIN ASSAY VALUES

Cereal	Number of samples	Mean protein, %	Procedure			
			Chemical		Microbiological	
			Niacin, μgm./gm.		Niacin, μgm./gm.	
			Mean	Range	Mean	Range
Wheat	23	14.0	60.8	50.2–78.1	59.6	45.7–72.3
Oats	28	12.7	13.5	9.5–22.6	12.1	8.0–18.7
Barley	24	13.8	62.1	48.9–74.3	71.2	58.7–87.3

A few assays were done on barley to compare results obtained chemically by a modification of the collaborative method described by Steele (10) and microbiologically on extracts treated with Lloyd's reagent, lead nitrate, and hydrogen peroxide as suggested by the reports of Brown, Thomas, and Bina (2, 3) who have demonstrated that treatment of extracts with hydrogen peroxide materially lowers the apparent niacin content of a number of materials as measured by microbiological methods.

The results shown in Columns 2 and 3 of Table II were obtained by the following procedures.

A two gram sample was weighed and suspended in 75 ml. of 2 *N* sulphuric acid in a 100 ml. volumetric flask. After hydrolysis in boiling water for one hour the flask was cooled and made up to volume with distilled water. The contents was well mixed and two 25 ml. aliquots, one for chemical and one for microbiological assay, were transferred to 50 ml. centrifuge tubes. Adsorption on Lloyd's reagent, elution with sodium hydroxide, and decolorization with lead nitrate were carried out for both tubes according to the method described by Steele (10). The niacin content of the extract from the first tube

was determined by the color development method of Melnick, Oser, and Siegel (9). Prior to microbiological assay the extract from the second tube was treated with 5 ml. of 40% sodium hydroxide and 5 ml. of 30% hydrogen peroxide at a temperature of 70° C. for two hours and then evaporated to dryness on a steam bath. The residue was dissolved in distilled water, adjusted to pH 6.8, and diluted to the original volume. The oxidized extract was assayed by the microbiological method of Krehl, Strong, and Elvehjem (7). The values obtained on three samples of barley are shown in Columns 2 and 3 of Table II. For comparison, the values obtained by the unmodified methods

TABLE II

BARLEY NIACIN ASSAY VALUES OBTAINED BY DIFFERENT METHODS

Sample	(1) Chemical, Melnick *et al.*	(2) Chemical following treatment with Lloyd's reagent and $Pb(NO_3)_2$	(3) Microbiological following treatment with Lloyd's reagent, $Pb(NO_3)_2$, and H_2O_2	(4) Microbiological, Krehl *et al.*
	μgm. per gm.			
B—1	52	34	36	63
B—2	66	40	45	77
B—3	68	43	48	75

of Melnick *et al.* and Krehl *et al.* are given in Columns 1 and 4. These results are in general agreement with those of Brown, Thomas, and Bina (3) who stress the importance of variations in apparent niacin values as determined by microbiological and chemical methods and point out the need for data on available niacin, derived from bioassays, to provide a sound basis for the interpretation of microbiological and chemical assay results.

The assay values given in the body of the present report were obtained by the microbiological method of Krehl, Strong, and Elvehjem (7). Although it is recognized that these values may not constitute an accurate measure of biologically available niacin in grains, particularly in wheat and barley, this method was adopted in preference to the chemical procedure of Melnick, Oser, and Siegel (9) owing to the fact that difficulty was experienced in obtaining reproducible results with different batches of cyanogen bromide, and in preference to methods involving adsorption and elution, because such methods are tedious for routine use.

Results

Comparative Niacin Content of Wheat, Barley, and Oats

The results of microbiological niacin assays done on 34 samples of Marquis wheat, 48 of Newal barley, and 50 of Victory oats grown under a wide variety of soil and climatic conditions in the province of Alberta during the years 1944 and 1945 are summarized in Table III.

TABLE III

NIACIN CONTENT OF GRAINS GROUPED ACCORDING TO YEAR

Year	No. of samples	% Protein, mean	Niacin, μgm./gm.	
			Mean	S. d.
Marquis wheat				
1944	20	12.9	50.8	7.6
1945	14	14.4	57.6	6.2
	34	13.5	53.6	7.8
Range			41.3 – 74.0	
Newal barley				
1944	26	12.4	69.9	11.3
1945	22	14.1	71.1	7.6
	48	13.2	70.4	9.7
Range			49.3 – 99.3	
Victory oats				
1944	26	10.9	8.7	1.9
1945	24	12.6	12.0	2.4
	50	11.7	10.3	2.7
Range			7.0 – 17.0	

When the extreme variations that exist in the niacin content of different samples of all three grains are taken into consideration, the mean niacin values of 53.6, 70.4, and 10.3 μgm. per gm. for wheat, barley, and oats respectively are in reasonably good agreement with those obtained on smaller numbers of samples by other workers (1, 4, 6, 11). Application of the *t* test to the present data showed that the difference between the mean niacin content of wheat and barley is highly significant, but in view of the uncertainty that exists (2, 3, 5) regarding the accuracy of microbiological niacin assay values as an index of biologically available niacin in these grains it is questionable whether barley should be ranked above wheat as a source of niacin. In this connection it may be noted that, as is shown in Table I, the mean niacin assay values for 23 samples of wheat and 24 samples of barley, as determined by the chemical procedure of Melnick, Oser, and Siegel (9), were approximately the same.

Niacin – Year Relations

It is shown in Table III that the mean niacin content of wheat samples grown in 1945 was approximately 13% higher than that of those grown in

1944. The corresponding figures for barley and oats are 2% and 38% respectively. Analysis of the data showed that for wheat and oats these differences are significant beyond the 1% point. While definite conclusions cannot be drawn from such limited data, the results suggest that significant variations may exist in the niacin content of wheat and oats grown in different years.

Niacin–Protein Relations

Inspection of Fig. 1 and Table IV shows that, although protein content was not a reliable index of the niacin content of wheat or oats, niacin and protein levels were found to be positively correlated in these grains. The

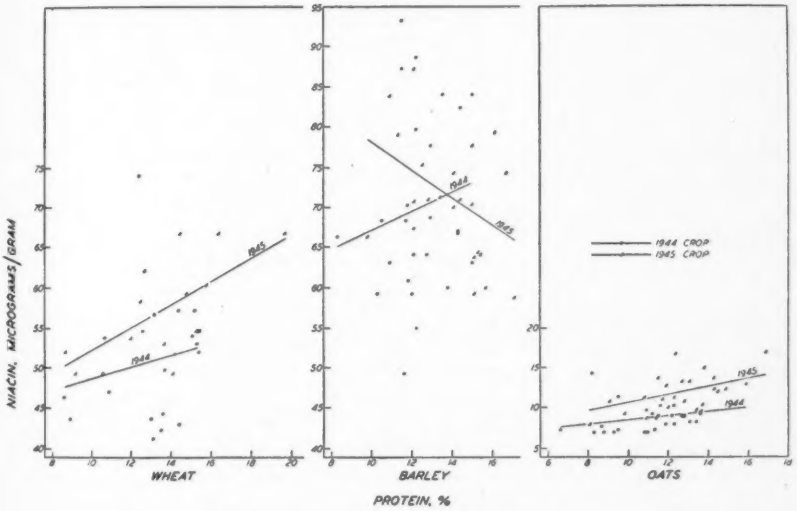

FIG. 1. *Relation between niacin and protein content of grains.*

TABLE IV

CORRELATION COEFFICIENTS BETWEEN NIACIN AND PROTEIN IN WHEAT, BARLEY, AND OATS

Cereal	Year	D. f.	r	5% pt.	1% pt.
Marquis wheat	1944	18	+.196	.444	.562
	1945	12	+.644*	.533	.662
	Both years	32	+.422*	.357	.437
Newal barley	1944	24	+.167	.388	.496
	1945	20	−.445*	.424	.536
	Both years	46	−.048	.285	.369
Victory oats	1944	24	+.298	.387	.496
	1945	22	+.410*	.403	.515
	Both years	48	+.488**	.279	.361

contrast between the niacin × protein correlation coefficients derived for Marquis wheat and Victory oats and those derived for Newal barley is interesting, and in this connection it may be mentioned that the niacin × protein correlation coefficient derived for eight samples of barley grown in 1946 was −0.206. Although neither this value nor the −0.048 coefficient for the total of 48 samples from the 1944 and 1945 crops is statistically significant, the results suggest that a tendency towards an inverse relation between niacin and protein may exist in barley.

Niacin – Soil Zone Relations

The effect of soil zone on the niacin content of wheat, barley, and oats was studied by grouping the data as summarized in Table V. It is apparent from the data in this table that differences in the mean niacin content of grains grown on different types of soil were relatively small and that the variability within any one soil zone was high. Analysis of the data showed that the difference between the mean of 57.4 μgm. of niacin per gram for nine samples of Marquis wheat grown on black or shallow black soil and the mean of 48.5 μgm. for nine samples grown on gray or gray transition soil is significant beyond the 1% point, but none of the other differences in mean niacin levels attributable to soil zone (Table V) were found to be statistically significant.

TABLE V

SUMMARY OF THE NIACIN CONTENT OF 1944 AND 1945 WHEAT, BARLEY, AND OAT SAMPLES, GROUPED ACCORDING TO SOIL ZONE

Year	Marquis wheat				Newal barley				Victory oats			
	No. samples	Mean protein, %	Niacin, μgm./gm.		No. samples	Mean protein, %	Niacin, μgm./gm.		No. samples	Mean protein, %	Niacin, μgm./gm.	
			Mean	S.d.			Mean	S.d.			Mean	S.d.
Gray and gray transition soil zone												
1944	7	10.9	46.9	3.6	6	11.0	71.5	10.1	9	9.4	8.4	2.5
1945	2	12.0	54.3	7.1	7	12.1	72.9	6.9	10	11.5	12.0	2.4
	9	11.1	48.5	5.2	13	11.6	72.3	8.2	19	10.5	10.3	3.0
Black and shallow black soil zone												
1944	6	13.8	59.0	7.5	10	13.0	73.5	11.3	9	11.7	8.4	1.0
1945	3	14.5	54.2	5.6	3	14.9	71.4	7.8	4	12.2	12.6	2.1
	9	14.0	57.4	6.9	13	13.4	73.0	10.6	13	11.9	9.7	2.4
Brown and dark brown soil zone												
1944	7	14.2	47.6	5.1	10	12.5	65.3	11.0	8	11.8	9.5	1.9
1945	9	14.1	59.5	6.1	12	15.0	70.0	8.8	10	13.8	11.7	2.8
	16	14.1	54.3	8.2	22	13.9	67.9	9.9	18	12.9	10.7	2.6

Discussion

From the present data for niacin in samples of wheat, barley, and oats and those reported by McElroy, Kastelic, and McCalla (8) for thiamine and riboflavin in the same samples, comparisons may be drawn between three species of grain and between three vitamins with respect to the degree of association that appears to exist between vitamin and protein levels in grains and between vitamin content and the type of soil on which grains are grown. The data indicate that although thiamine and protein levels tend to be positively correlated in all three grains the relation is much closer in wheat and oats than in barley. It would appear that niacin levels also tend to vary directly with protein levels in wheat and oats, while in barley there is some evidence that an inverse relation may exist between niacin and protein. The data for riboflavin fail to demonstrate the existence of any correlation between levels of this vitamin and protein in wheat, barley, or oats.

Variations in vitamin levels that may be associated with soil zones seem to be more marked for thiamine than for either riboflavin or niacin. Although some of the differences were small the mean thiamine content of all three grains was found to be highest in samples grown on brown soils, intermediate in those grown on black soils, and lowest in those from the gray soil zone. The data for riboflavin – soil zone relations are less conclusive, although they do provide some evidence to suggest that in wheat and oats riboflavin levels may tend to be slightly lower in samples grown on gray soils than in those grown in either black or brown soil zones. In the case of niacin, no uniform trend that would suggest the existence of a close association between soil type and levels of this vitamin in grains was observed.

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References

1. BARTON-WRIGHT, E. C. Biochem. J. 38 : 314-319. 1944.
2. BROWN, E. B., THOMAS, J. M. and BINA, A. F. Cereal Chem. 20 : 201-211. 1943.
3. BROWN, E. B., THOMAS, J. M. and BINA, A. F. J. Biol. Chem. 162 : 221-228. 1946.
4. DAVIS, C. F., LAUFER, S. and SALETAN, L. Cereal Chem. 20 : 109-113. 1943.
5. HALE, E. B., DAVIS, G. K. and BALDWIN, H. R. J. Biol. Chem. 146 : 553-563. 1942.
6. HALE, E. B., DAVIS, G. K. and BALDWIN, H. R. J. Biol. Chem. 146 : 565-570. 1942.
7. KREHL, W. A., STRONG, F. M. and ELVEHJEM, C. A. Ind. Eng. Chem., Anal. Ed. 15 : 471-475. 1943.
8. MCELROY, L. W., KASTELIC, J. and MCCALLA, A. G. Can. J. Research, F, 26 : 191-200. 1948.
9. MELNICK, D., OSER, B. L. and SIEGEL, L. Ind. Eng. Chem., Anal. Ed. 13 : 879-883. 1941.
10. STEELE, H. K. Cereal Chem. 22 : 448-454. 1945.
11. TEPLY, L. J., STRONG, F. M. and ELVEHJEM, C. A. J. Nutrition 23 : 417-423. 1942.